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(54) Title: COMPOSITIONS AND METHODS FOR PROMOTING IMMUNOSUPPRESSION

(57) Abstract: The invention provides compositions and methods for promoting immunosuppression by administering a chemokine binding protein, in combination with an immunosupressant.

COMPOSITIONS AND METHODS FOR PROMOTING IMMUNOSUPPRESSION

Field of the Invention

This invention relates to immunosuppression and inflammation.

Background of the Invention

Certain immunological disorders are caused, in part, by a severe immune response to either the body's own tissue (*e.g.*, autoimmune disorders) or to foreign substances (*e.g.*, bacteria, viruses, transplanted organs). These disorders are generally accompanied by a inflammatory response, which is the body's reaction to tissue injury.

A number of viral proteins specifically counteract or subvert the development of the host inflammatory response and acquired cellular immunity, and poxviruses, in general, are a rich source of anti-inflammatory proteins (Turner *et al.*, *Cur. Top. Microbiol. Imm.*, 163:125-152, 1990; Buller *et al.*, *Micro. Dev.*, 55:80-122, 1991; Smith, *J. Gen. Virol.*, 94:1725-1740, 1993; McFadden, G., (Ed.), "*Viroceptors, virokines and related immune modulators encoded by DNA viruses*," *R.G. Landes Co.*, Austin Texas, 1995). Examples of such viral proteins include myxoma growth factor (MGF), Serp 1, M11L, and the chemokine binding proteins M-T7 (CBP-1), M-T1 (CBP-2), and p35.

In addition, many immunological disorders are treated using compounds that suppress the immune response (immunosuppressants). However, traditional immunosuppressive techniques, using standard immunosuppressants such as cyclosporine, are associated with severe side effects, including nausea, vomiting, hair loss, nephrotoxicity, and an increased

risk of infection and of developing malignancies, particularly when administered at the high doses that are required for maximal efficacy.

In view of the significant numbers of people affected by immunological disorders, it would be beneficial to provide effective and safe long-term therapies for the treatment of these disorders.

Summary of the Invention

The invention provides compositions and methods for promoting immunosuppression by administering a chemokine binding protein, in combination with an immunosupressant.

In one aspect, the invention provides a method for promoting immunosuppression in a mammal, by administering to the mammal a therapeutically-effective amount of a chemokine binding protein, or biologically-active fragment or analog of the chemokine binding protein, and an immunosuppressant. In a preferred embodiment, the mammal has an autoimmune disorder, such as rheumatoid arthritis, psoriasis, atopic dermatitis, bronchial asthma, pollinosis, systemic lupus erythematosus, nephrotic syndrome lupus, multiple sclerosis, myasthenia gravis, type I and type II diabetes mellitus, uveitis, glomerulonephritis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, and AIDS.

In a second aspect, the invention provides a method for the treatment of transplant rejection of an organ, by administering to a mammal a therapeutically-effective amount of a chemokine binding protein, or biologically-active fragment or analog thereof, and an immunosuppressant. In a preferred embodiment of the second aspect, the organ is a heart, liver, lung, bone marrow, cornea, pancreas, intestinum tenue, limb, spleen, muscle, nerve, fatty marrow, duodenum, blood, skin, or pancreatic islet cell. In another preferred embodiment of the second aspect, the transplant rejection is chronic

or acute.

In preferred embodiments of the first two aspects, the chemokine binding protein is administered at a dosage of between 0.1 μg/kg -10,000 μg/kg, in a single dose or in multiple doses. In other preferred embodiments of the first two aspects, the chemokine binding protein is administered by injection and the immunosuppressant is administered orally, or the chemokine binding protein and the immunosuppressant are administered by co-injection. In another preferred embodiment of the first two aspects, the effect of the chemokine binding protein and the immunosuppressant is synergistic

In a third aspect, the invention provides a pharmaceutical composition for promoting immunosuppression, including a therapeutically-effective amount of a chemokine binding protein, or biologically-active fragment or analog thereof, in combination with an immunosuppressant, in a pharmaceutically-acceptable carrier.

In preferred embodiments of all three aspects, the chemokine binding protein is M-T7, M-T1, or p35, and the immunosuppressant is cyclosporine, FK501, mycophenolate mofetil, azathioprine, cyclophosphamide, methotrexate, mizoribine, rapamycin (sirolimus), antithymocyte immunoglobulin, corticosteroids, or tacrolimus. In other preferred embodiments of all three aspects, the immunosuppressant is an anti-rejection agent.

By "chemokine binding protein" is meant a polypeptide that binds to and inhibits a chemokine. Chemokines are small molecular weight ligands which are chemoattractants for leukocytes (*e.g.*, neutrophils, basophils, monocytes, and T cells), and are believed to be critical for infiltration of lymphocytes and monocytes into sites of inflammation. Exemplary chemokine binding proteins are M-T7 (CBP-1, described in U.S. Patent No. 5, 834,419, PCT publication WO 96/33730, and indicated herein as SEQ ID NOs: 1-4); M-T1 (CBP-2, described in PCT publication WO 97/44054 and indicated herein

as SEQ ID NOs: 5-8); and p35 (described in PCT publications WO 98/37217, and WO 97/11714, and U.S. Patent Nos. 5, 834,419, and 5, 871, 740), incorporated by reference herein. Chemokine binding proteins are useful for treating a variety of inflammatory and immunological disorders associated with the trafficking of lymphocytes and monocytes from the circulation to tissue sites during inflammation and immune responses to damage, infection and various disease states.

By "biologically active fragment" is meant a polypeptide fragment of a chemokine binding protein that exhibits chemokine binding and chemokine inhibitory properties that are at least 30%, preferably at least 50%, more preferably at least 75%, and most preferably at least 100%, compared with the chemokine binding and chemokine inhibitory properties of a full length chemokine binding protein. By "analog" is meant any substitution, addition, or deletion in the amino acid sequence of a chemokine binding protein that exhibits properties that are at least 30%, preferably at least 50%, more preferably at least 75%, and most preferably at least 100%, compared with the chemokine binding and chemokine inhibitory properties of a chemokine binding protein from which it is derived. Fragments and analogs can be generated using standard techniques, for example, solid phase peptide synthesis or polymerase chain reaction. The properties of a chemokine binding protein can be determined using standard techniques known to those skilled in the art, and also described in, for example, PCT publications WO 96/33730 and WO 97/44054.

By "treating" is meant the medical management of a patient with the intent that a cure, amelioration, or prevention of a disease, pathological condition, or disorder will result. This term includes active treatment, that is, treatment directed specifically toward improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the disease, pathological condition, or

disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventive treatment, that is, treatment directed to prevention of the disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the disease, pathological condition, or disorder. The term "treating" also includes symptomatic treatment, that is, treatment directed toward constitutional symptoms of the disease, pathological condition, or disorder.

By "therapeutically-effective amount" is meant an amount of a chemokine binding protein and an immunosuppressant sufficient to produce a healing, curative, prophylactic, stabilizing, or ameliorative effect in the treatment of an immunological disorder (*e.g.*, autoimmune diseases, transplant rejection).

By "pharmaceutically acceptable carrier" is meant a carrier that is physiologically acceptable to the treated mammal while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline solution. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in *Remington: The Science and Practice of Pharmacy*, (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, PA.

By "immunological disorder" is meant any disease which involves the immune response or immunity in general. More specifically, an immunological disorder is a malfunction of the immune system that reduces the ability of an organism to resist foreign substances in the body (*e.g.*, viruses, bacteria, bacterial toxins, plant pollen, fungal spores, animal danders, medications, foods, allogeneic or xenogeneic transplanted organs) or causes the body to produce antibodies against its own tissues (*e.g.*, autoimmune

disorders), resulting in tissue injury. Immunological disorders can also occur when a malfunctioning immune system (caused by, for example, genetic defect, illness, injury, malnutrition, medications such as those used for chemotherapy) results in an increase in frequency or severity of infections. Immunological disorders are often accompanied by inflammation, which is the body's reaction to tissue injury, and results in the accumulation of white blood cells, macrophages, and lymphocytes at the site of injury. The term "anti-inflammatory" refers to reduction or suppression of an inflammatory response.

By "transplant rejection" is meant an acute or chronic decrease in the physiological function of a transplanted organ, as measured by biological factors specific to the organ transplanted. For example, to assess the rejection of a kidney transplant, increases in glomerular atrophy, intimal thickening, tubular atrophy, interstitial fibrosis, lymphocyte infiltration, cortical scarring, serial serum creatinine levels, graft survival prolongation, hyalinization, and other indicia known to those skilled in the art, are considered independently or together, as indicators of graft rejection. Similarly, to assess the rejection of a heart transplant, increased cardiac vessel disease post-transplant, and increased graft intimal hyperplasia, compared to control grafted vessels, for example, are considered independently or together, as indicators of graft rejection.

By "immunosuppressant" is meant a compound that suppresses the immune response by, for example, removing immune cells from the immune system or inhibiting the ability of immune cells to respond to cytokines.

By "anti-rejection agent" is meant a compound that reduces the tendency of a transplanted organ to be rejected by the transplant recipient. Anti-rejection agents include immunosuppressants.

By "promoting immunosuppression" is meant an effect (*e.g.*, physiological effect) that results in an increase in immunosuppression of at least 1.5-fold, preferably at least 2-fold, more preferably at least 3-fold, and most preferably over 5-fold, upon administration of a chemokine binding

protein, in combination with an immunosuppressant, relative to administration of an immunosuppressant alone.

By "synergy" or "synergistic" is meant the cooperative interaction of two compounds such that the combined effect of the two compounds is greater than the additive effect of each compound administered in the absence of the other.

Brief Description of the Drawings

Figure 1 is a histogram showing the histopathological endpoints for control (CsA alone) vs M-T7 or SERP treated rats.

Figure 2 is a photograph showing the tubulo-interstitial histology of control (CsA alone) vs M-T7 or SERP treated rats.

Figure 3 is a photograph showing the glomerular histology of control (CsA alone) vs M-T7 or SERP treated rats.

Figure 4 is a photograph showing the vascular histology of control (CsA alone) vs M-T7 or SERP treated rats.

Figure 5 is a photograph showing the cortical histology of control (CsA alone) vs M-T7 or SERP treated rats.

Figure 6A-B shows the nucleotide and amino acid sequences of a M-T7 protein (CBP-1) (SEQ ID NOs: 1-2).

Figure 7A-B shows the nucleotide and amino acid sequences of a M-T7 protein (CBP-1) (SEQ ID NOs: 3-4).

Figure 8 shows the nucleotide and amino acid sequences of a M-T1 protein (CBP-2) (SEQ ID NOs: 5-6).

Figure 9 shows the nucleotide and amino acid sequences of a M-T1 protein (CBP-2) (SEQ ID NOs: 7-8).

Detailed Description of the Invention

The invention provides compositions and methods for promoting immunosuppression, for example, for the treatment of immunological disorders. To this end, the invention features the use of a chemokine binding protein, or a biologically-active fragment or analog of a chemokine binding protein, in combination with an immunosuppressant, to promote immunosuppression and treat immunological disorders (*e.g.*, autoimmune disorders, transplant rejection).

We have discover that a chemokine binding protein, when used in conjunction with an immunosuppressant, is surprisingly more efficacious in the treatment of an immunological disorder, such as transplant rejection, than either the chemokine binding protein or the immunosuppressant alone. For example, co-administration of a chemokine binding protein and an immunosuppressant for a period of about 1 to about 30 days post-transplant, is efficacious to treat graft rejection without further administration of an immunosuppressant. The synergistic effect of the active components of the present invention drastically improves both short term and long term treatment outcome.

Administration of Chemokine Binding Proteins and Immunosuppressants

Conventional pharmaceutical practice is employed to provide suitable formulations or compositions for simultaneous, separate, or sequential administration to patients. Oral administration of the immunosuppressant and intravenous administration of the chemokine binding protein is preferred, but any other appropriate route of administration may be employed, for example, parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, or aerosol administration. Therapeutic formulations may be in the form of liquid solutions, microemulsions, or suspensions (as, for

example, for intravenous administration); for oral administration, formulations may be in the form of liquids, tablets, or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are described, for example, in "*Remington: The Science and Practice of Pharmacy*" (19th ed.) ed. A.R. Gennaro, 1995, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes.

If desired, slow release or extended release delivery systems may be utilized. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel. Preferably, chemokine binding proteins are administered at a dosage of between 0.1 μg/kg -10,000 μg/kg, and more preferably between 1 μg/kg - 1000 μg/kg. In general, chemokine binding proteins are administered at a dosage appropriate to the effect to be achieved. Immunosuppressants are administered at standard dosages recommended by the manufacturers, and known to one skilled in the art. The exact dosage of the chemokine binding protein and the immunosuppressant is likely to be dependent, for example, upon the age and weight of the recipient, the route of administration, and the severity and nature of the condition or symptoms to be treated. In general, the dosage selected should be sufficient to promote immunosuppression, or prevent, ameliorate, or treat an immunological

disorder, or one or more symptoms thereof, without producing significant toxic or undesirable side effects. As noted herein, the preferred route of administration for most indications is oral and/or by injection.

The chemokine binding protein and the immunosuppressant of the invention can be administered as a single dose, or can be administered in multiple doses. Furthermore, the chemokine binding protein can be administered separately from the immunosuppressant. For example, the chemokine binding protein can be administered by injection, while the immunosuppressant can be administered orally. The period of administration of the chemokine binding protein can be different from the period of administration of the immunosuppressant, for example, in cases where the immunosuppressant is administered immediately after an allograft to prevent acute rejection, and the chemokine binding protein is co-administered later to prevent chronic rejection. Accordingly, the immunosuppressant can be administered prior to (*e.g.*, 1 to 30 days) co-administration of the chemokine binding protein. The chemokine binding protein and the immunosuppressant of the invention can be administered continuously for a period of time, for example, every day for a number of days or months.

In the case of transplantation, a single bolus or multiple doses can be administered to the recipient and/or the donor before, at the time of, or subsequent to implanting the organ in the recipient. The particular protocol will depend on the nature of the organ, and the type of chemokine binding protein and immunosuppressant used.

Subjects

The subject of the invention is preferably a human, however, any mammal with an immunological disorder can be treated using the compositions and methods of the invention. For example, subjects may include non-human primates, domestic animals (*e.g.*, sheep, horses, cattle, pigs), and experimental

animals (*e.g.*, rats, mice, hamsters). In addition, organs derived from a subject may also be used according to the present invention. In the case of transplantation, the donor organs may have 0, 1, 2, or more HLA mismatches with the recipient.

Immunological Disorders

Any immunological disorder than would benefit by treatment with an immunosuppressant or anti-inflammatory agent is within the scope of this invention. Immunological disorders within the scope of this invention include, without limitation, autoimmune disorders, immunodeficiency disorders, and the immunological response to organ or tissue transplantation.

Autoimmune disorders, where the immune system attacks the host's own tissues, include, but are not limited to, inflammatory bowel disease, dermatitis, meningitis, thrombotic thrombocytopenic purpura, Sjögren's syndrome, encephalitis, leukocyte adhesion deficiency, rheumatoid and other forms of immune arthritis, rheumatic fever, Reiter's syndrome, psoriatic arthritis, progressive systemic sclerosis, primary biliary cirrhosis, pemphigus, pemphigoid, necrotizing vasculitis, rheumatoid arthritis, atopic dermatitis, bronchial asthma, pollinosis, systemic lupus erythematosus, nephrotic syndrome lupus, multiple sclerosis, myasthenia gravis, type I and type II diabetes mellitus, glomerulonephritis, Hashimoto's thyroiditis, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune hemolytic anemia, Graves disease, habitual spontaneous abortions, Reynard's syndrome, dermatomyositis, chronic active hepatitis, celiac disease, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

Other immunological disorders within the scope of this invention include, without limitation, immunodeficiency disorder (AIDS); toxic shock syndrome; endotoxemia or septic shock (sepsis); artherosclerotic plaque growth; post-reperfusion injury; restenosis; ultraviolet and radiation responses; non-malignant or immunological-related cell-proliferative diseases such as psoriasis, pemphigus vulgaris, Behcet's syndrome; acute respiratory distress syndrome (ARDS); ischemic heart disease; atherosclerosis; post-dialysis syndrome; leukemia; lipid histiocytosis; disorders associated with the activation of T cells, B cells, macrophages, and other inflammatory leukocytes during the immune response and the acute phase response; disorders associated with advanced cancer, such as tumor necrosis factor-mediated cachexia; and allergic disorders, such as allergic rhinitis, asthma, and food allergies.

Organs or tissues for transplantation include, without limitation, heart, liver, lung, bone marrow, cornea, pancreas, intestinum tenue, limb, spleen, muscle, nerve, fatty marrow, duodenum, blood, blood vessel, artery, skin, and pancreatic islet cell. Both allogeneic transplantation and xenotransplantation are included within the scope of this invention.

The following examples are provided for the purpose of illustrating the invention and should not be construed as limiting.

EXAMPLE 1

M-T7, in Combination with Cyclosporine (CsA), Prevented Chronic Allograft Rejection in a Orthotopic Rat Kidney Model

M-T7, in combination with Cyclosporine (CsA), provided effective prevention of kidney rejection as measured by a significant decrease of the accepted histopathological signs and symptoms of kidney organ rejection, when compared to M-T7 alone and CsA alone (described below). Histopathological endpoint measurements included tubular atrophy, glomerular atrophy, vascular hyalinization, vascular intimal thickening, and cortical

scarring.

A rat model of chronic kidney transplantation was used. This model is a functional kidney transplant model, where the kidney of a donor rat is transplanted to a recipient rat whose kidneys are removed prior to receiving the new kidney. Orthotopic kidney transplants, from F344 male rats to Lewis rats, were performed. The recipients' native kidneys were removed at the time of surgery and the animals received subtherapeutic doses of CsA (0.75 mg/kg subcutaneously) for 10 days to prevent initial acute rejection. Animal survival depended solely on the proper functioning of the transplanted kidney. Under these conditions, more than 80% of animals were expected to survive to post-operative day (POD) 140 and to exhibit features of chronic renal allograft rejections (such as intimal thickening, glomerular sclerosis, tubular atrophy, and interstitial as well as cortical fibrosis). These animals were included in the final analysis.

The protocol used was as follows:

Group 1: F344 to Lewis with CsA + vehicle (PBS/saline); n= 10 (control);

Group 2: F344 to Lewis with CsA + M-T7 low dose (500X = 800 pg/g); n= 10;

Group 3: F344 to Lewis with CsA + M-T7 medium dose (5,000X = 8 ng/g); n= 10;

Group 4: F344 to Lewis with CsA + M-T7 high dose (50,000X = 80 ng/g); n= 10;

Group 5: Lewis to Lewis isografts; n=10;

Group 6: F344 to F344 isografts.

M-T7 was given daily, in combination with CsA (0.75 mg/kg sc), starting at POD 0 (day of transplant) and ending POD 9 (10 injections total). The route of administration was intravenous (IV) through the tail vein. The dose ranged from a low dose of 800 pg/g, a medium dose of 8 ng/g, and a high dose of 80 ng/g. The following outcome variables were assessed: 1) serial

serum creatinine levels on POD 140; 2) CsA levels on POD 7; 3) routine immunopathology on POD 140. Sera and kidneys were frozen for further analysis. Conditions for M-T7 administration were identical to those for SERP-1 (U.S. Provisional Patent Application No. 60/161,643, filed October 27, 1999), incorporated by reference.

Table 1 shows the survival, CsA levels, and renal function (as assessed by creatinine levels) in control and treated rats at low, medium, and high doses of M-T7, and indicates that the combination of M-T7 and CsA is more effective at preventing transplant rejection, using accepted indicia, than CsA alone. The comparable or higher CsA levels in the Allograft CsA 0.75 mg/kg (experimental) box vs. the Allograft CsA 0.75 mg/kg + low, medium, and high dose M-T7 boxes, indicates that renal function was improved in M-T7/CsA-treated rats even in the presence of lower levels of CsA.

Microscopic Hematoxylin-Eosin (HE) slides and tricrone stained slides were read to assess histopathological changes at necropsy. The severity of chronic rejection was scored as: 0 = no changes; 1 = minimal changes; 2 = mild changes; 3 = moderate changes; and 4 = marked changes. The median score of histopathological changes in control and treated rats at low, medium, and high doses of M-T7, and percentage of positive cases are shown in Table 2. Figure 1 shows a histogram depicting the efficacy of M-T7 in transplant rejection, as measured by histopathological changes in control and treated rats, while Figures 2-5 show photographs of the histopathology of selected tissues of control and treated rats.

Renal allografts in the control group developed typical chronic rejection characterized by tubular atrophy (Figure 2), glomerular atrophy (Figure 3), vascular endothelitis (Figure 4), and cortical scarring (Figure 5). In contrast, there were minimal changes in the renal allografts treated with the combination of CsA and a short course of M-T7 (Figures 1-5, Table 2). These results indicate that the combination of M-T7 and CsA is synergistically more

effective at reducing injury and improving renal function and histology than CsA alone, thus preventing chronic transplant rejection in this model.

EXAMPLE 2

Assessment of a Chemokine Binding Protein, in Combination with an Immunosuppressant, in a Heterotopic Heart Transplantation Model in MHC Mismatched Rats

The most commonly used animal model for preclinical research on graft vascular disease is one in which a heterotopic heart transplantation is performed in MHC mismatched rats. In this model, rats treated with only CsA for the first 7 days after transplantation develop graft vascular disease when analyzed at POD 90. The PVG to ACI strain combination is used, and the recipient is treated with 7.5 mg/kg Neoral® (Novartis Pharma AG, Basel, Switzerland) per gavage from day 0 to day 9. The incidence of acute rejection (and consequent loss of the animal) is 30%, and the average luminal narrowing is 50% at POD 90. This model provides a powerful system for the assessment of the efficacy of a chemokine binding protein (*e.g.*, M-T7, M-T1, or p35), in combination with an immunosuppressant, for treating transplant rejection.

The pulmonary veins and the venae cava are tied off at the time of graft harvest in the donor animal. The heart is perfused with a preservation solution, and then immediately implanted into the recipient animal. This is accomplished by anastomizing the donor aorta to the recipient abdominal aorta, and the donor pulmonary artery to the recipient vena cava. The vascular clamps are then removed, and the donor heart starts beating following reperfusion, thus creating a primary vascularized, non-working heart transplantation model. Graft function is monitored by daily palpation. The quality of graft function is scored on a scale of 0 to 4, where 0 is a non-beating heart allograft and 4 is a vigorously beating heart allograft. Acute rejection is diagnosed clinically if the palpation score is less than 1. Subacute rejection, in

this model, is defined if the palpation score is less than or equal to 2, with histological evidence of massive inflammatory infiltration. The recipient animals are followed for 90 days, at which time the animals are sacrificed and the heart is excised. Thin hematoxylin/eosin stained sections of paraffin-embedded samples are assessed by a pathologist, blinded to the treatment regiment, for the presence of graft vascular disease. The individual vessels are scored based on a five-point grading scale, where 0 = no involvement, 1 = partial intimal involvement, 2 = concentric intimal thickening, 3 = severe concentric involvement with up to 50% luminal narrowing, and 4 = more than 50% luminal narrowing. In addition, the lesions are quantified by morphometric analysis, and the morphometrically assessed score and mean compared between the individual experimental groups.

The experimental groups are as follows:

Group 1: Neoral® monotherapy (7.5 mg/kg POD 0-9; Control);

Group 2: Neoral® (7.5 mg/kg POD 0-9) + M-T7 low dose (500X = 800 pg/g POD 0-9);

Group 3: Neoral® (7.5 mg/kg POD 0-9) + M-T7 medium dose (5,000X = 8 ng/g POD 0-9);

Group 4: Neoral® (7.5 mg/kg POD 0-9) + M-T7 high dose (50,000X = 80 ng/g POD 0-9);

Group 5: Neoral® (7.5 mg/kg POD 0-9) + M-T7 high dose (50,000X = 80 ng/g POD 0-9); Readministration of Neoral® (7.5 mg/kg) + M-T7 high dose (50,000X = 80 ng/g) POD 30 and 60.

The animals are followed for 90 days, and then sacrificed. Graft palpation and weight are assessed during the follow-up period. Graft palpation score is assessed as 4 = strong beating, 3 = moderately beating, 2 = weakly beating, 1 = not beating. Weight is assessed during the days of drug treatment and at the time of sacrifice, and expressed as percentage weight change in comparison to the weight at the time of surgery.

Morphometric analysis is performed as follows. Each heart is cut horizontally along the long-axis of the graft and 4 sections were with HE. All coronary arteries are identified in each section and analyzed separately by morphometry. Morphometric analysis includes determination of the free, unoccluded vessels lumen, the vessel area inside the basal membrane (intima plus free lumen, and the total vessel area.

Morphometric outcome variables are: (a) number of diseased vessels, where disease is defined as any noticeable amount of intima; and (b) percent intimal area; vessel area inside the basal membrane minus the free lumen divided by the vessel area inside the basal membrane.

EXAMPLE 3

Assessment of a Chemokine Binding Protein, in Combination with an Immunosuppressant, in a Heterotopic Mouse Heart Transplantation Model

The efficacy of a chemokine binding protein (*e.g.*, M-T7, M-T1, or p35), in combination with an immunosuppressant, for the treatment of acute and chronic transplant rejection is assessed in a heterotopic mouse heart allograft transplant model.

The heterotopic mouse heart allograft transplant model is performed as previously described (Zhang *et al.*, *Transplantation* 62:1267-72, 1996). Briefly, a median sternotomy is performed in the donor, and the right and left superior vena cavae are ligated. The ascending aorta and pulmonary artery of the donor are anastomosed end to side to the recipient aorta and inferior vena cava, respectively.

For the acute rejection model, male inbred mice C57BL/6($H2^b$) and BALB/c($H2^d$) are used as the donor and the recipient, respectively. This strain combination is mismatched in both major and minor MHC, and rejects the graft 9 days after grafting (*Zhang et* al., *Transplantation* 62:1267-72, 1996). Direct palpation of heart grafts is performed daily. Complete cessation of

cardiac impulses is considered as the end point of rejection, and the animal is sacrificed and necropsy performed. Lymphocyte infiltration, vasculitis, infarction, ischemia, and thrombosis are among the criteria that can be used to assess rejection. Animals are followed until the development of end point rejection, or sacrifice at 30 days, for acute rejection.

The experimental groups are as follows

Group 1: CsA 20 mg/kg IM POD 0=7, then every other day until day 28;

Group 2: CsA 20 mg/kg IM POD 0=7, then every other day until day 28 + M-T7 low dose (500X = 800 pg/g) IV POD 0, 2, 4, & 7;

Group 3: CsA 20 mg/kg IM POD 0=7, then every other day until day 28 + M-T7 medium dose (5,000X = 8 ng/g) IV POD 0, 2, 4, & 7;

Group 4: CsA 20 mg/kg IM POD 0=7, then every other day until day 28 + M-T7 high dose (50,000X = 80 ng/g) IV POD 0, 2, 4, & 7;

Group 5: M-T7 low dose (500X = 800 pg/g) IV POD 0, 2, 4, & 7;

Group 6: M-T7 high dose (50,000X = 80 ng/g) IV POD 0, 2, 4, & 7;

Group 7: no treatment.

EXAMPLE 4

Assessment of a Chemokine Binding Protein, in Combination with an Immunosuppressant, in a Rat Skin Allograft Model

A rat skin allograft survival assay, using MHC-incompatible rat strains as donor and acceptor has been described (Inamura *et al.*, *Transplantation* 45:206-9, 1988). WKAH donor and F344 recipient rats are selected, and full thickness skin grafts (2.0×2.0 cm^2) are transplanted to the lateral thorax of recipients and wrapped in sterile, bactericidal gauze. The chest is then wrapped with an elastic bandage. Five days after transplantation, the wraps are removed and the grafts inspected daily for rejection. Rejection is defined as more than 90% necrosis of graft epithelium. This skin allograft model can be used to assess the efficacy of a chemokine binding protein (*e.g.*, M-T7, M-T1,

or p35), in combination with an immunosuppressant, for treating skin transplant rejection.

EXAMPLE 5

Assessment of a Chemokine Binding Protein, in Combination with an Immunosuppressant, in a Mouse Systemic Lupus Erythematosus Model

The efficacy of a chemokine binding protein (*e.g.*, M-T7, M-T1, or p35), in combination with an immunosuppressant, for the treatment of systemic lupus erythematosus (SLE) is assessed in a mouse model. NZB/W F1 hybrid mice spontaneously generate an immune pathology that is clinically and immunologically similar to human SLE. This mouse model of SLE is highly predictive and can be used as a preclinical model for assessing new treatments for SLE. This animal model is characterized by substantial proteinuria, by the presence of autoantibodies, and by the growth of glomerulonephritis, the principle cause of death in these animals. Immunosuppressants such as cyclophosphamide and prednisone are capable of delaying the pathology.

EXAMPLE 6

Assessment of a Chemokine Binding Protein, in Combination with an Immunosuppressant, in Animal Models of Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is an animal model for the study of multiple sclerosis, an autoimmune disease directed against antigens of the central nervous system. The acute (early) phase of EAE is followed by a remission phase, after which there is a chronic phase consisting of alternating relapses and remissions. EAE can be used to assess the efficacy of a chemokine binding protein, in combination with an immunosuppressant, for the treatment of multiple sclerosis.

Treatment experiments in EAE can be performed in Lewis rats after active immunization and adoptive cell transfer (Rott *et al.*, *Eur. J. Immunol.* 23:1745-51, 1993). In clinically manifest EAE, treatment is started within 6 hours of the onset of symptoms. To distinguish between a long-term prophylactic or a temporary suppressive effect, animals receive a chemokine binding protein and an immunosuppressant from the day of active immunization until day 11. Histological analysis is performed on animals with EAE to detect cellular infiltrates.

In another animal model, SJL/J mice are transfused on day 0 with T lymphocytes, primed with myelin basic protein, to induce EAE. Twenty-four hours later on day 1, and every day thereafter until day 10, the mice are subcutaneously injected either with an immunosuppressant (as a control) or with varying amounts of a chemokine binding protein and an immunosuppressant (five mice per experimental or control group). The chemokine binding protein-treated mice are disease-free and symptom-free at the time that treatment is initiated; as is well known by those who use this animal model of MS, signs of EAE do not appear until 6-10 days after the disease-causing T lymphocytes are transferred into the mice.

Other Embodiments

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come

within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the appended claims.

Other embodiments are within the claims.

What is claimed is:

Claims

1. A method for promoting immunosuppression in a mammal, comprising administering to said mammal a therapeutically-effective amount of a chemokine binding protein, or biologically-active fragment or analog thereof, and an immunosuppressant.

2. A method for the treatment of transplant rejection of an organ, comprising administering to a mammal a therapeutically-effective amount of a chemokine binding protein, or biologically-active fragment or analog thereof, and an immunosuppressant.

3. A pharmaceutical composition for promoting immunosuppression, comprising a therapeutically-effective amount of a chemokine binding protein, or biologically-active fragment or analog thereof, in combination with an immunosuppressant, in a pharmaceutically-acceptable carrier.

4. The method of claim 1, wherein said mammal has an autoimmune disorder.

5. The method of claim 4, wherein said autoimmune disorder is selected from the group consisting of rheumatoid arthritis, psoriasis, atopic dermatitis, bronchial asthma, pollinosis, systemic lupus erythematosus, nephrotic syndrome lupus, multiple sclerosis, myasthenia gravis, type I and type II diabetes mellitus, uveitis, glomerulonephritis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, and AIDS.

6. The method of claims 1, 2, and 3, wherein said chemokine binding protein is selected from the group consisting of M-T7, M-T1, and p35.

7. The method of claims 1, 2, and 3, wherein said immunosuppressant is selected from the group consisting of cyclosporine, FK501, mycophenolate mofetil, azathioprine, cyclophosphamide, methotrexate, mizoribine, rapamycin (sirolimus), antithymocyte immunoglobulin, corticosteroids, and tacrolimus.

8. The method of claim 2, wherein said organ is selected from the group consisting of heart, liver, lung, bone marrow, cornea, pancreas, intestinum tenue, limb, spleen, muscle, nerve, fatty marrow, duodenum, blood, skin, and pancreatic islet cell.

9. The method of claims 1 and 2, wherein said chemokine binding protein is administered at a dosage of between 0.1 μg/kg -10,000 μg/kg.

10. The method of claims 1 and 2, wherein said chemokine binding protein is administered in a single dose.

11. The method of claims 1 and 2, wherein said chemokine binding protein is administered in multiple doses.

12. The method of claims 1 and 2, wherein said chemokine binding protein is administered by injection and said immunosuppressant is administered orally.

13. The method of claims 1 and 2, wherein said chemokine binding protein and said immunosuppressant are administered by co-injection.

14. The method of claims 1, 2, and 3, wherein said immunosuppressant is an anti-rejection agent.

15. The method of claim 2, wherein said transplant rejection is chronic.

16. The method of claim 2, wherein said transplant rejection is acute.

17. The method of claims 1 and 2, wherein the effect of said chemokine binding protein and said immunosuppressant is synergistic.

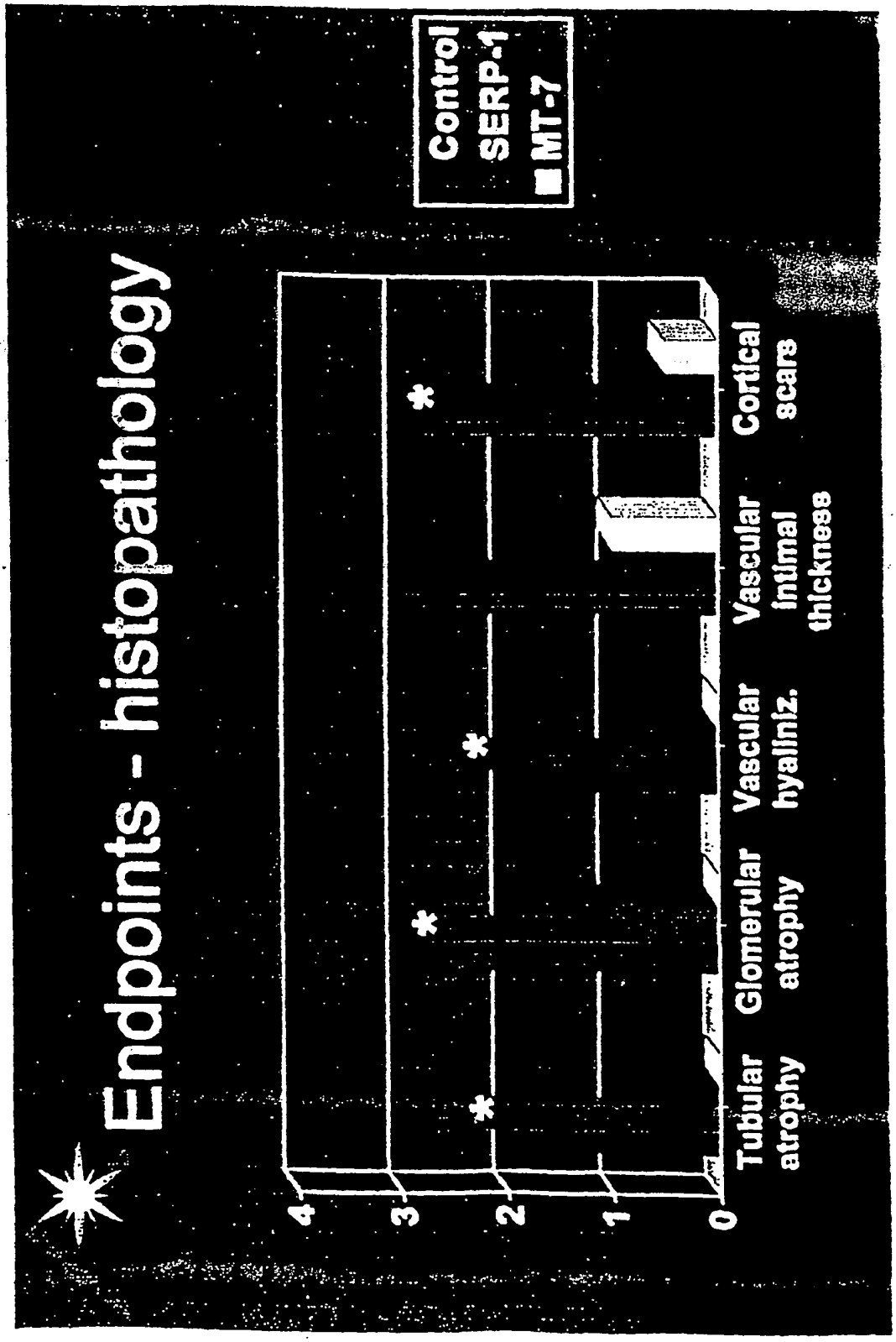

Endpoints - histopathology
Control
SERP-1
MT-7
4
3
2
1
0
*
*
*
Tubular atrophy
Glomerular atrophy
Vascular hyalinlz.
Vascular intimal thickness
Cortical scars

FIGURE 1

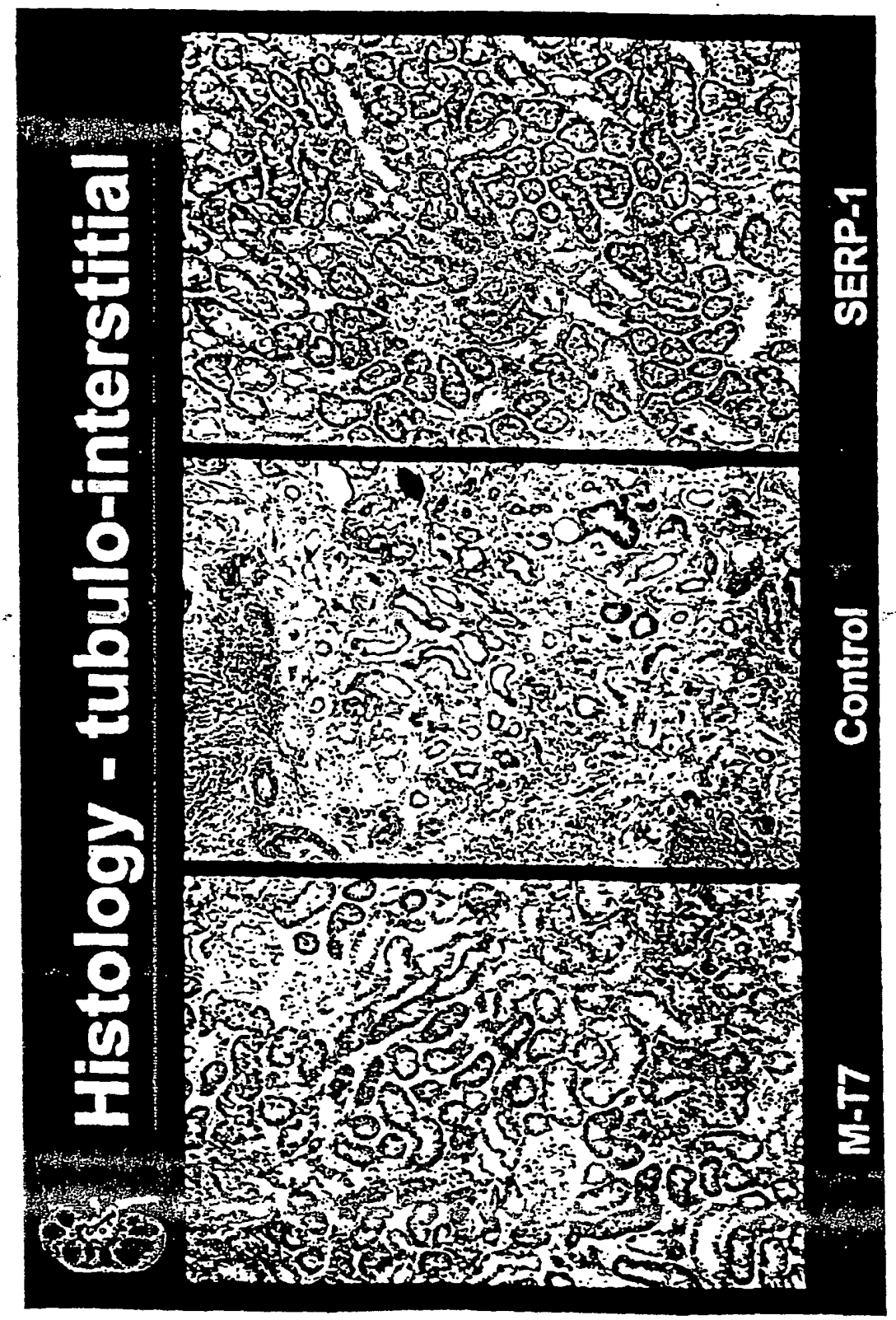
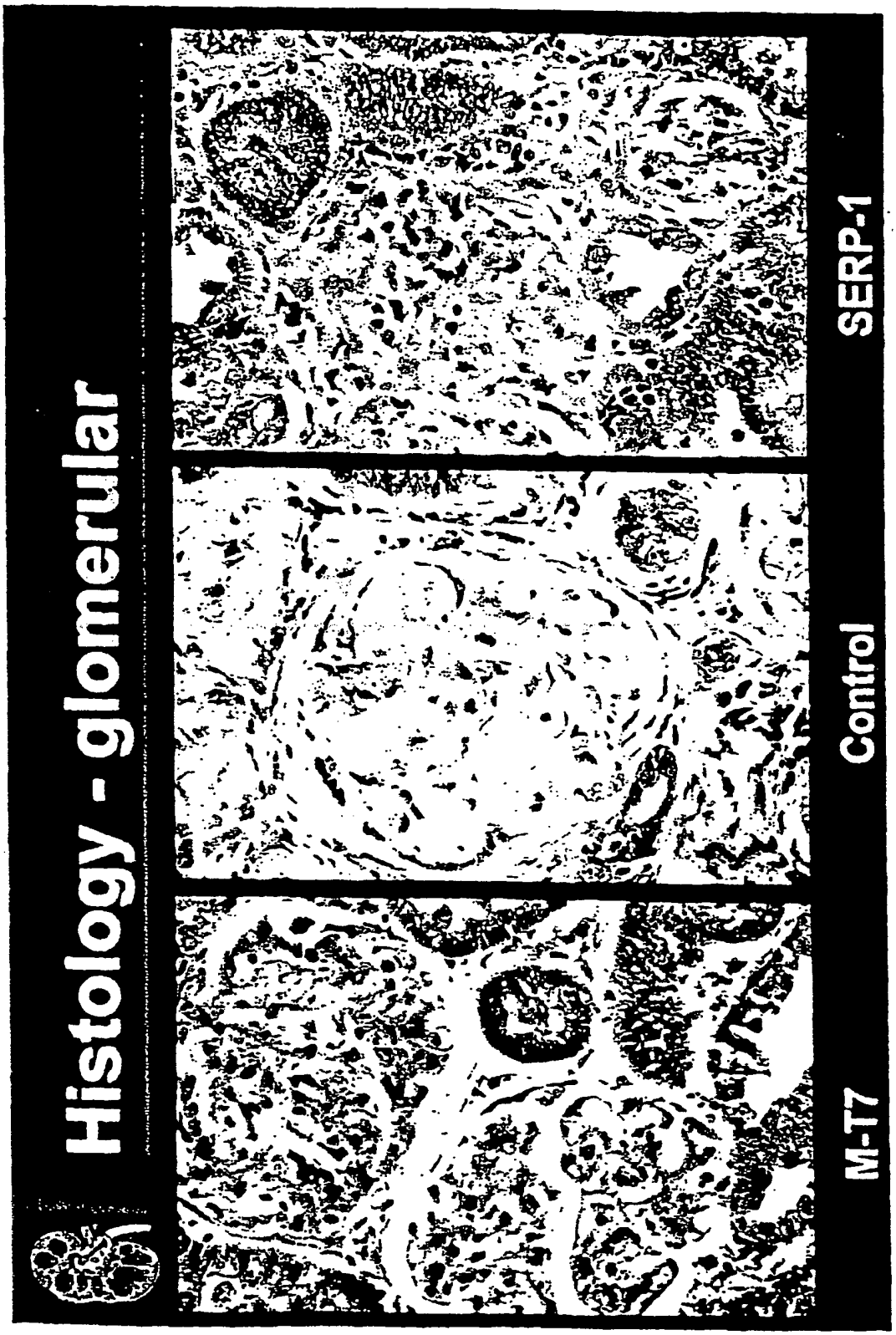
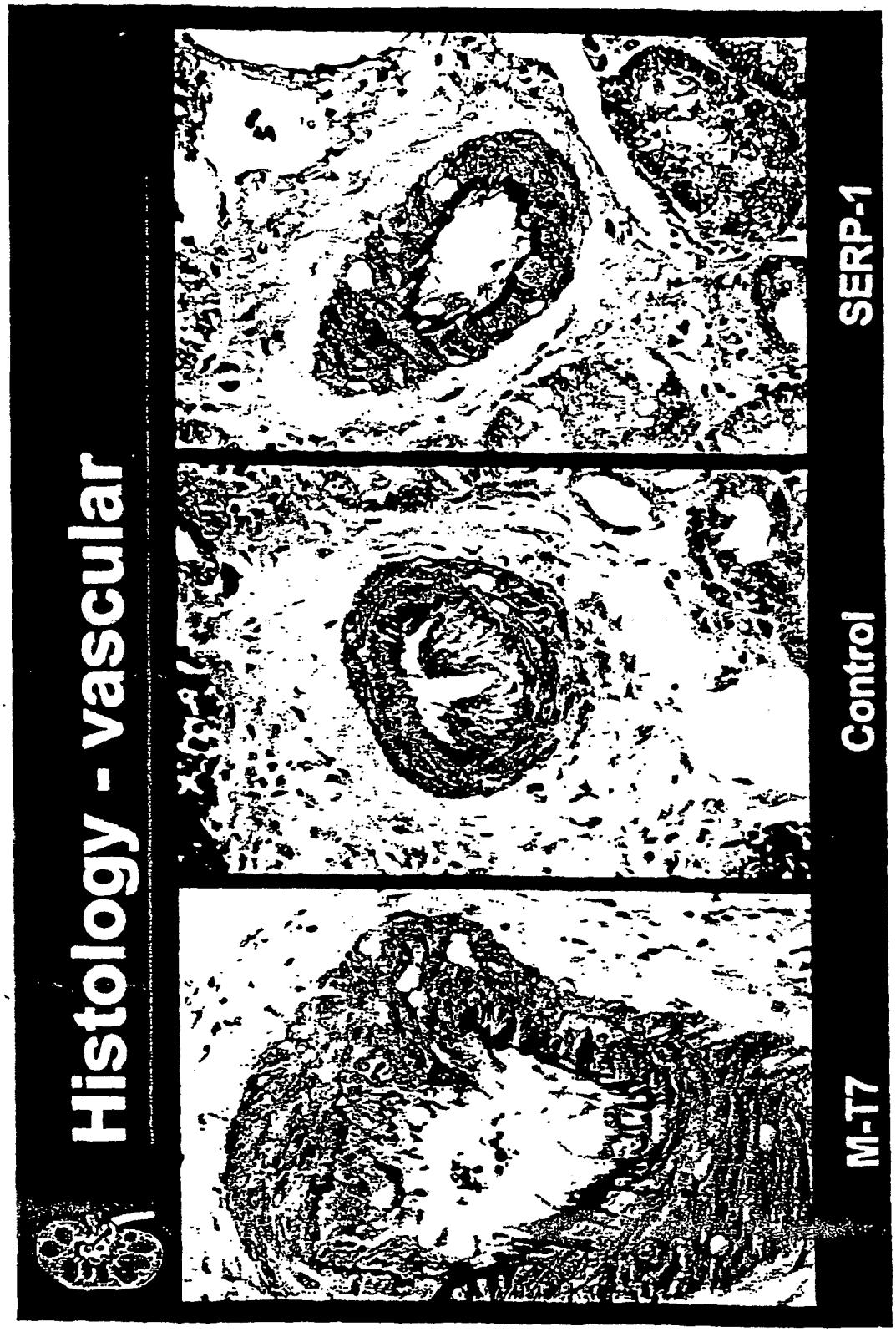


FIGURE 2


Histology - glomerular
M-T7
Control
SERP-1


Histology - vascular
M-T7
Control
SERP-1

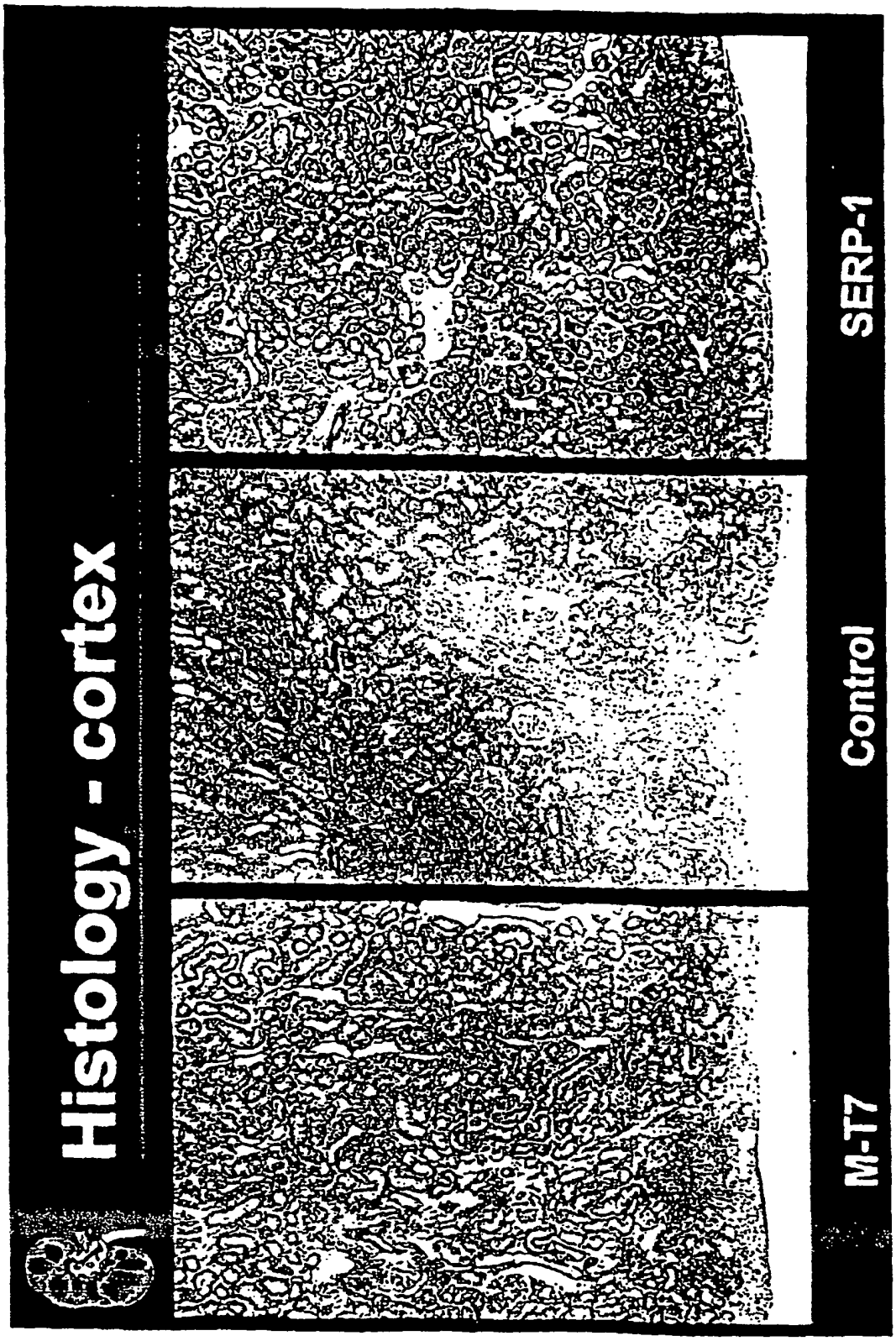


FIGURE 5

```
GGATCCATCG AGACGGCGTC CCGGACGTCA CGGACTTCGT TCAGAAACTA TCCGGAGGTA      60

CATGGACGAA GGTGAACGAA CTGTCCGTCC CCAAGGCGAG CGTTACGGCG ATCGTCTATA     120

AAGAGAGGTT GTACTGCGTA GGGGGGCTGG TGGATCGATA CGCTCCAACG AACGAAGTTA     180

TCCGTTACAG GGACGACACG AACGAGTGGG AATACGTGGG ATCTACGAAG ATCGAACGAG     240

GCGGTTCCGT GGGGTGTGTG TACAACGACG AGCTCTACGT CTTCGGAGGA ACGGATACGT     300

TTACGTCCGA GCGATACAAC GGAGTCATTT GGAAACGAGC GAACGACGTC TCCTGTCACT     360

TCGCCACCAT GAACGCGGCG TACGCCACCT ACCTCGAGCT GTAGAAACGT TTTTATAACT     420

GAAAAAGTAT CCTAAAAATA GAGTAATACT CAAG ATG GAC GGG AGA CTG GTG          472
                                      Met Asp Gly Arg Leu Val
                                        1               5

TTT CTC CTC GCG TCG CTC GCT ATC GTC TCC GAC GCC GTA CGC CTT ACG        520
Phe Leu Leu Ala Ser Leu Ala Ile Val Ser Asp Ala Val Arg Leu Thr
            10                  15                  20

TCC TAC GAC TTA AAC ACA TTC GTT ACG TGG CAA GAC GAT GGA TAC ACC        568
Ser Tyr Asp Leu Asn Thr Phe Val Thr Trp Gln Asp Asp Gly Tyr Thr
        25                  30                  35

TAC AAC GTC AGT ATT AAA CCG TAT ACG ACG GCT ACG TGG ATC AAT GTG        616
Tyr Asn Val Ser Ile Lys Pro Tyr Thr Thr Ala Thr Trp Ile Asn Val
    40                  45                  50

TGT GAA TGG GCG TCT TCT AGC TGC AAC GTA TCT CTC GCC CTA CAA TAC        664
Cys Glu Trp Ala Ser Ser Ser Cys Asn Val Ser Leu Ala Leu Gln Tyr
55                  60                  65                  70

GAT TTG GAC GTC GTG TCT TGG GCC AGA CTG ACC CGG GTT GGT GGG TAC        712
Asp Leu Asp Val Val Ser Trp Ala Arg Leu Thr Arg Val Gly Gly Tyr
                75                  80                  85

ACA GAA TAC AGT CTG GAA CCG ACG TGT GCC GTG GCT CGG TTC TCT CCA        760
Thr Glu Tyr Ser Leu Glu Pro Thr Cys Ala Val Ala Arg Phe Ser Pro
            90                  95                  100

CCG GAG GTA CAA CTC GTA AGA ACA GGT ACC AGC GTA GAA GTC TTA GTT        808
Pro Glu Val Gln Leu Val Arg Thr Gly Thr Ser Val Glu Val Leu Val
        105                 110                 115

AGA CAC CCC GTC GTG TAT CTA CGG GGG CAG GAA GTG TCC GTC TAC GGA        856
Arg His Pro Val Val Tyr Leu Arg Gly Gln Glu Val Ser Val Tyr Gly
    120                 125                 130

CAT TCA TTC TGC GAC TAC GAC TTC GGG TAT AAA ACG ATC TTC CTG TTC        904
His Ser Phe Cys Asp Tyr Asp Phe Gly Tyr Lys Thr Ile Phe Leu Phe
135                 140                 145                 150

TCG AAG AAT AAA CGA GCG GAG TAC GTC GTA CCC GGC CGA TAT TGC GAC        952
Ser Lys Asn Lys Arg Ala Glu Tyr Val Val Pro Gly Arg Tyr Cys Asp
                155                 160                 165
```

FIGURE 6A

```
AAC GTA GAG TGT CGT TTC TCC ATC GAT TCC CAA GAA AGT GTA TGT GCT      1000
Asn Val Glu Cys Arg Phe Ser Ile Asp Ser Gln Glu Ser Val Cys Ala
            170                 175                 180

ACG GCG GTT CTT ACG TAC GGT GAC AGT TAT CGT TCC GAG GCG GGT GTG      1048
Thr Ala Val Leu Thr Tyr Gly Asp Ser Tyr Arg Ser Glu Ala Gly Val
        185                 190                 195

GAG GTC TGC GTT CCC GAA CTC GCG AAG AGA GAA GTC AGT CCC TAC ATC      1096
Glu Val Cys Val Pro Glu Leu Ala Lys Arg Glu Val Ser Pro Tyr Ile
    200                 205                 210

GTG AAA AAG TCG TCC GAC CTG GAA TAC GTC AAA CGT GCC ATA CAC AAC      1144
Val Lys Lys Ser Ser Asp Leu Glu Tyr Val Lys Arg Ala Ile His Asn
215                 220                 225                 230

GAA TAC CGA CTC GAC ACC TCC TCC GAG GGA CGC AGA TTG GAG GAA CTG      1192
Glu Tyr Arg Leu Asp Thr Ser Ser Glu Gly Arg Arg Leu Glu Glu Leu
                235                 240                 245

TAT CTA ACG GTC GCC TCC ATG TTT GAA CGT CTC GTG GAA GAT GTC TTC      1240
Tyr Leu Thr Val Ala Ser Met Phe Glu Arg Leu Val Glu Asp Val Phe
            250                 255                 260

GAA TAATCGAAAT ATAAATAATG TAGTTTTTGT ATCGGAATCA TGGAACGTAC           1293
Glu  (SED ID NO: 2)


CCTGGTAAGT TTCTTGGACA GCGGTACCAT GAGCGACATC ACCCTCGTCG CGGGGGAGAC    1353

GTCGTTCACG GCGCATCGAC TGATTTTATC CGTCCATTCG GATTACTTCT ATCGTCTGTT    1413

TAACGGGAGT TTTGAGGTAC CGGATACGAT CACGTTGGAT ACGGACGATG GCGTCCTTCG    1473

CACCGTGCTC CGCTACATGT ACACGGGATA CAGCAACATA CGAGACCGTA CCGTAGAGGA    1533

TCTACAATCC ATTATCGTAT TGGCGGACTA CCTGGGTATA ACGAAACTGG TGAAAGAGTG    1593

TGCGGATTAC ATGGTAAGTC GAGTGGACCC GACGAACTGC GTATCCGCTT TCCAGTTTGC    1653

AGAGACGTAT CACATAGAGG ATTTAAAACG AAACCTCAAT ACGTTCTTAC CCGAACTCTT    1713

GCTGAACTCC CGAGGGGCGT TTACGAAATT GGATACGGAC GAAGCGGTCG TGGTTCTACG    1773

AGCGTCCTAC GAGATCGTCG ACAGACGGTT TGTGCTTAAG GCTATTCTAG ATTGGGTGCG    1833

AAAAGGACCC AAACGCATCG AGCGGATAAA GACGCTGTCC GCGG (SED ID NO: 1)      1877
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FIGURE 6B

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GGATCCATCG AGACGGCGTC CCGGACGTCA CGGACTTCGT TCAGAAACTA TCCGGAGGTA      60

CATGGACGAA GGTGAACGAA CTGTCCGTCC CCAAGGCGAG CGTTACGGCG ATCGTCTATA     120

AAGAGAGGTT GTACTGCGTA GGGGGGCTGG TGGATCGATA CGCTCCAACG AACGAAGTTA     180

TCCGTTACAG GGACGACACG AACGAGTGGG AATACGTGGG ATCTACGAAG ATCGAACGAG     240

GCGGTTCCGT GGGGTGTGTG TACAACGACG AGCTCTACGT CTTCGGAGGA ACGGATACGT     300

TTACGTCCGA GCGATACAAC GGAGTCATTT GGAAACGAGC GAACGACGTC TCCTGTCACT     360

TCGCCACCAT GAACGCGGCG TACGCCACCT ACCTCGAGCT GTAGAAACGT TTTTATAACT     420

GAAAAAGTAT CCTAAAAATA GAGTAATACT CAAG ATG GAC GGG AGA CTG GTG          472
                                      Met Asp Gly Arg Leu Val
                                        1               5

TTT CTC CTC GCG TCG CTC GCT ATC GTC TCC GAC GCC GTA CGC CTT ACG        520
Phe Leu Leu Ala Ser Leu Ala Ile Val Ser Asp Ala Val Arg Leu Thr
            10                  15                  20

TCC TAC GAC TTA AAC ACA TTC GTT ACG TGG CAA GAC GAT GGA TAC ACC        568
Ser Tyr Asp Leu Asn Thr Phe Val Thr Trp Gln Asp Asp Gly Tyr Thr
        25                  30                  35

TAC AAC GTC AGT ATT AAA CCG TAT ACG ACG GCT ACG TGG ATC AAT GTG        616
Tyr Asn Val Ser Ile Lys Pro Tyr Thr Thr Ala Thr Trp Ile Asn Val
    40                  45                  50

TGT GAA TGG GCG TCT TCT AGC TGC AAC GTA TCT CTC GCC CTA CAA TAC        664
Cys Glu Trp Ala Ser Ser Ser Cys Asn Val Ser Leu Ala Leu Gln Tyr
55                  60                  65                  70

GAT TTG GAC GTC GTG TCT TGG GCC AGA CTG ACC CGG GTT GGT AAA TAC        712
Asp Leu Asp Val Val Ser Trp Ala Arg Leu Thr Arg Val Gly Lys Tyr
                75                  80                  85

ACA GAA TAC AGT CTG GAA CCG ACG TGT GCC GTG GCT CGG TTC TCT CCA        760
Thr Glu Tyr Ser Leu Glu Pro Thr Cys Ala Val Ala Arg Phe Ser Pro
            90                  95                  100

CCG GAG GTA CAA CTC GTA AGA ACA GGT ACC AGC GTA GAA GTC TTA GTT        808
Pro Glu Val Gln Leu Val Arg Thr Gly Thr Ser Val Glu Val Leu Val
        105                 110                 115

AGA CAC CCC GTC GTG TAT CTA CGG GGG CAG GAA GTG TCC GTC TAC GGA        856
Arg His Pro Val Val Tyr Leu Arg Gly Gln Glu Val Ser Val Tyr Gly
    120                 125                 130

CAT TCA TTC TGC GAC TAC GAC TTC GGG TAT AAA ACG ATC TTC CTG TTC        904
His Ser Phe Cys Asp Tyr Asp Phe Gly Tyr Lys Thr Ile Phe Leu Phe
135                 140                 145                 150

TCG AAG AAT AAA CGA GCG GAG TAC GTC GTA CCC GGC CGA TAT TGC GAC        952
Ser Lys Asn Lys Arg Ala Glu Tyr Val Val Pro Gly Arg Tyr Cys Asp
                155                 160                 165
```

FIGURE 7A

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AAC GTA GAG TGT CGT TTC TCC ATC GAT TCC CAA GAA AGT GTA TGT GCT      1000
Asn Val Glu Cys Arg Phe Ser Ile Asp Ser Gln Glu Ser Val Cys Ala
            170                 175                 180

ACG GCG GTT CTT ACG TAC GGT GAC AGT TAT CGT TCC GAG GCG GGT GTG      1048
Thr Ala Val Leu Thr Tyr Gly Asp Ser Tyr Arg Ser Glu Ala Gly Val
        185                 190                 195

GAG GTC TGC GTT CCC GAA CTC GCG AAG AGA GAA GTC AGT CCC TAC ATC      1096
Glu Val Cys Val Pro Glu Leu Ala Lys Arg Glu Val Ser Pro Tyr Ile
    200                 205                 210

GTG AAA AAG TCG TCC GAC CTG GAA TAC GTC AAA CGT GCC ATA CAC AAC      1144
Val Lys Lys Ser Ser Asp Leu Glu Tyr Val Lys Arg Ala Ile His Asn
215                 220                 225                 230

GAA TAC CGA CTC GAC ACC TCC TCC GAG GGA CGC AGA TTG GAG GAA CTG      1192
Glu Tyr Arg Leu Asp Thr Ser Ser Glu Gly Arg Arg Leu Glu Glu Leu
                235                 240                 245

TAT CTA ACG GTC GCC TCC ATG TTT GAA CGT CTC GTG GAA GAT GTC TTC      1240
Tyr Leu Thr Val Ala Ser Met Phe Glu Arg Leu Val Glu Asp Val Phe
            250                 255                 260

GAA TAATCGAAAT ATAAATAATG TAGTTTTTGT ATCGGAATCA TGGAACGTAC           1293
Glu  (SED ID NO: 4)


CCTGGTAAGT TTCTTGGACA GCGGTACCAT GAGCGACATC ACCCTCGTCG CGGGGGAGAC    1353

GTCGTTCACG GCGCATCGAC TGATTTTATC CGTCCATTCG GATTACTTCT ATCGTCTGTT    1413

TAACGGGAGT TTTGAGGTAC CGGATACGAT CACGTTGGAT ACGGACGATG GCGTCCTTCG    1473

CACCGTGCTC CGCTACATGT ACACGGGATA CAGCAACATA CGAGACCGTA CCGTAGAGGA    1533

TCTACAATCC ATTATCGTAT TGGCGGACTA CCTGGGTATA ACGAAACTGG TGAAAGAGTG    1593

TGCGGATTAC ATGGTAAGTC GAGTGGACCC GACGAACTGC GTATCCGCTT TCCAGTTTGC    1653

AGAGACGTAT CACATAGAGG ATTTAAAACG AAACCTCAAT ACGTTCTTAC CCGAACTCTT    1713

GCTGAACTCC CGAGGGGCGT TTACGAAATT GGATACGGAC GAAGCGGTCG TGGTTCTACG    1773

AGCGTCCTAC GAGATCGTCG ACAGACGGTT TGTGCTTAAG GCTATTCTAG ATTGGGTGCG    1833

AAAAGGACCC AAACGCATCG AGCGGATAAA GACGCTGTCC GCGG (SED ID NO: 3)      1877
```

FIGURE 7B

```
1       TACAGCGACAGTAATCATCCCGAGGAGGTCGACGACTTCGTGGAATACCATTGGGGTACA

61      CGCCTCCGTCTCTTTCCCTCACCCAAACGATGTAGACTCGTTTCATAGATTACGGATTTT

121     CTTCTAGTTAAATTCTTAAAAAAAAAGTCGAATTATAATAAAACGTGGGCGTATAGAAGAA

181     CTCTATCATGAAACGCCTGTGTGTATTATTCGCGTGCCTGGCCGCGACCCTCGCGACGAA
              M  K  R  L  C  V  L  F  A  C  L  A  A  T  L  A  T  K

241     GGGCATCTGCAGACAAGGCGAAGATGTCCGATACATGGGAATAGACGCCGTGGCCAAAAT
         G  I  C  R  Q  G  E  D  V  R  Y  M  G  I  D  A  V  A  K  I

301     TACAAAGAGGACTACCGGAAGCGACACGCCGTGTCAGGGTCTGCGTACGACTATTGAATC
         T  K  R  T  T  G  S  D  T  P  C  Q  G  L  R  T  T  I  E  S

361     CGCGTATACAGAAGACGAAAACGAAGACGATGGCGCGACGGGTACGGAGCAGCCCGACGA
         A  Y  T  E  D  E  N  E  D  D  G  A  T  G  T  E  Q  P  D  D

421     TCTTAGCGAGGAATACGAGTACGACGAAAACGACGAATCGTTTCTAACCGGTTTCGTGAT
         L  S  E  E  Y  E  Y  D  E  N  D  E  S  F  L  T  G  F  V  I

481     CGGAAGTACTTACCACACGATCGTCGGAGGAGGACTCTCCGTCACGTTCGGATTTACGGG
         G  S  T  Y  H  T  I  V  G  G  G  L  S  V  T  F  G  F  T  G

541     ATGTCCTACCGTTAAGGCGATATCCGAACACGTCAAAGGACGCCACGTCTACGTCCGACT
         C  P  T  V  K  A  I  S  E  H  V  K  G  R  H  V  Y  V  R  L

601     GTCCAGCGACGCTCCTTGGAGAGATACGAATCCCGTGTCTATGAACCGTACAGAGGCGCT
         S  S  D  A  P  W  R  D  T  N  P  V  S  M  N  R  T  E  A  L

661     CGCCCTACTCGACACGTGTGAAGTGTCCGTAGATATCAAATGCAGTCGCGTCAACGTAAC
         A  L  L  D  T  C  E  V  S  V  D  I  K  C  S  R  V  N  V  T

721     CGAAACGACGTACGGAACCGCGGCGCTTGTCCCGCGTATAACTCAAGCGACGAGACGCAG
         E  T  T  Y  G  T  A  A  L  V  P  R  I  T  Q  A  T  R  R  S

781     TCATATTATCGGATCTACCCTGGTCGACACGGAATGTGTGAAGAGTCTAGACATAACCGT
         H  I  I  G  S  T  L  V  D  T  E  C  V  K  S  L  D  I  T  V

841     CCAAGTGGGTGAAATGTGTAAGAGAACGTCTGATCTCTCGGCGAGAGACAGTCTTAAGGT
         Q  V  G  E  M  C  K  R  T  S  D  L  S  A  R  D  S  L  K  V

901     AAAGAACGGCAAACTACTCGAGGACGATATCCTTGTCCTTCGTACGCCTACCCTCAAGGC
         K  N  G  K  L  L  E  D  D  I  L  V  L  R  T  P  T  L  K  A

961     GTGTAACTAATCCTATCTACGATCGATGTCGTATTTTTCTGACCGTTACGCGTCACGTTT
         C  N  (SEQ ID NO: 6)

1021    TTATACCTATATAAAYAGKTAAAACCCATATAGGGAATACCGCTCGCTTTTTTTTCCTTC

1081    GTAGTTGTTTACCCGCTCGATAGATCGCGTCGAGGAAGTACCAACCGTGACCACTCCTCC

1141    GGCGGGGATCC (SEQ ID NO:5)
```

FIGURE 8

```
1       TACAGCGACAGTAATCATCCCGAGGAGGTCGACGACTTCGTGGAATACCATTGGGGTACA

61      CGCCTCCGTCTCTTTCCCTCACCCAAACGATGTAGACTCGTTTCATAGATTACGGATTTT

121     CTTCTAGTTAAATTCTTAAAAAAAAGTCGAATTATAATAAAACGTGGGCGTATAGAAGAA

181     CTCTATCATGAAACGCCTGTGTGTATTATTCGCGTGCCTGGCCGCGACCCTCGCGACGAA
              M  K  R  L  C  V  L  F  A  C  L  A  A  T  L  A  T  K

241     GGGCATCTGCAGACAAGGCGAAGATGTCCGATACATGGGAATAGACGTCGTGGCCAAAAT
         G  I  C  R  Q  G  E  D  V  R  Y  M  G  I  D  V  V  A  K  I

301     TACAAAGAGGACTACCGGAAGCGACACGCCGTGTCAGGGTCTGCGTACGACTATTGAATC
         T  K  R  T  T  G  S  D  T  P  C  Q  G  L  R  T  T  I  E  S

361     CGCGTATACAGAAGACGAAAACGAAGACGATGGCGCGACGGGTACGGAGCAGCCCGACGA
         A  Y  T  E  D  E  N  E  D  D  G  A  T  G  T  E  Q  P  D  D

421     TCTTAGCGAGGAATACGAGTACGACGAAAACGACGAATCGTTTCTAACCGGTTTCGTGAT
         L  S  E  E  Y  E  Y  D  E  N  D  E  S  F  L  T  G  F  V  I

481     CGGAAGTACTTACCACACGATCGTCGGAGGAGGACTCTCCGTCACGTTCGGATTTACGGG
         G  S  T  Y  H  T  I  V  G  G  G  L  S  V  T  F  G  F  T  G

541     ATGTCCTACCGTTAAGGCGATATCCGAACACGTCAAAGGACGCCACGTCTACGTCCGACT
         C  P  T  V  K  A  I  S  E  H  V  K  G  R  H  V  Y  V  R  L

601     GTCCAGCGACGCTCCTTGGAGAGATACGAATCCCGTGTCTATGAACCGTACAGAGGCGCT
         S  S  D  A  P  W  R  D  T  N  P  V  S  M  N  R  T  E  A  L

661     CGCCCTACTCGACACGTGTGAAGTGTCCGTAGATATCAAATGCAGTCGCGTCAACGTAAC
         A  L  L  D  T  C  E  V  S  V  D  I  K  C  S  R  V  N  V  T

721     CGAAACGACGTACGGAACCGCGGCGCTTGTCCCGCGTATAACTCAAGCGACGAGACGCAG
         E  T  T  Y  G  T  A  A  L  V  P  R  I  T  Q  A  T  R  R  S

781     TCATATTATCGGATCTACCCTGGTCGACACGGAATGTGTGAAGAGTCTAGACATAACCGT
         H  I  I  G  S  T  L  V  D  T  E  C  V  K  S  L  D  I  T  V

841     CCAAGTGGGTGAAATGTGTAAGAGAACGTCTGATCTCTCGGCGAGAGACAGTCTTAAGGT
         Q  V  G  E  M  C  K  R  T  S  D  L  S  A  R  D  S  L  K  V

901     AAAGAACGGCAAACTACTCGAGGACGATATCCTTGTCCTTCGTACGCCTACCCTCAAGGC
         K  N  G  K  L  L  E  D  D  I  L  V  L  R  T  P  T  L  K  A

961     GTGTAACTAATCCTATCTACGATCGATGTCGTATTTTTCTGACCGTTACGCGTCACGTTT
         C  N  (SEQ ID NO: 10)

1021    TTATACCTATATAAAYAGKTAAAACCCATATAGGGAATACCGCTCGCTTTTTTTTCCTTC

1081    GTAGTTGTTTACCCGCTCGATAGATCGCGTCGAGGAAGTACCAACCGTGACCACTCCTCC

1141    GGCGGGGATCC (SEQ ID NO:7)
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FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/16029

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/00

US CL :514/2; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HOFFMAN et al. Chemokine regulation of CNS T-cell infiltration in experimental autoimmune encephalomyelitis. Research in Immunology. 1998, Vol. 149, No. 9, pages 790-794, see entire article.	1, 3, 4, 5
Y	ELSNER et al. Exotaxin-2 activates chemotaxis-related events and release of reactive oxygen species via pertussis toxin-sensitive G proteins in human eosinophils. Eur. J. Immunol. 1998, Vol. 28, pages 2152-2158, see entire article.	1, 3, 4, 5
Y	WO 97/44054 A2 (UNIVERSITY OF ALBERTA) 27 November 1997, see entire document, especially Abstract, pages 13-15, and claims.	1-5, 8, 15, 16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 28 JULY 2001	Date of mailing of the international search report 14 AUG 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARIANNE DIBRINO Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/16029

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,834,419 A (MCFADDEN ET AL) 10 November 1998, see entire document, especially Abstract, column 6 at lines 31-56 and claims.	1, 3, 4, 5
Y	WO 96/33730 A2 (THE GOVERNORS OF THE UNIVERSITY OF ALBERTA) 31 October 1996, see entire document, especially pages 12-15 and claims.	1, 2, 3, 4, 5, 8, 15, 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/16029

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6, 7, 9-14 AND 17
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/16029

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST 2.O, STN(EMBASE, BIOSIS, MEDLINE, CAPLUS, SCISEARCH)
search terms: inventors' names, chemokine binding protein/s, m-t7, m-t1, p35, immunosuppress/ion, transplant/ation, cyclosporine, rapamycin, methotrexate, corticosteroids chemokine, administer